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Phytic acid enhances biocontrol efficacy of *Rhodotorula mucilaginosa* against postharvest gray mold spoilage and natural spoilage of strawberries

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ABSTRACT

The control activity of *Rhodotorula mucilaginosa*, alone or in combination with phytic acid (PA) on gray mold spoilage and natural spoilage of strawberries was investigated. *R. mucilaginosa* as stand-alone treatment significantly reduced the disease incidence of gray mold spoilage of strawberries at 20 °C, and the combination of *R. mucilaginosa* and PA at the concentration of 4 mol/ml and 6 mol/ml were more effective than *R. mucilaginosa* alone treatment. *In vitro* test showed that PA at the concentration of 4 mol/ml and 6 mol/ml significantly enhanced the inhibition of the growth of *Botrytis cinerea* on PDA. PA at all the tested concentrations enhanced the growth of *R. mucilaginosa* in NYDB media. PA at the concentration of 4 mol/ml slightly increased the population growth of R. mucilaginosa in strawberry wounds at the first day at 20 °C, and slightly increased the population growth of *R. mucilaginosa* in fruit wounds at 4 °C at the whole storage time. The combination of *R. mucilaginosa* and PA at the concentration of 4 mol/ml was the most effective treatment in controlling the natural spoilage of strawberries following storage at 4 °C for 20 d followed by 20 °C for 5 d.

1. Introduction

Strawberry fruit have a very short postharvest life, often estimated in less than 5 days. It is very prone to rapid dehydration, physiological disorders, bruising and other mechanical injuries, and to infections caused by several pathogens that can rapidly reduce quality of ripe fruits (Sallato, Torres, Zoffoli, & Latorre, 2007). Gray mold spoilage caused by *Botrytis cinerea* is one of the most destructive postharvest diseases of strawberries (Romanazzi, Nigro, Ippolito, & Salerno, 2001; Wszelaki & Mitcham, 2003). The shelf-life of the strawberry is very short because of its perishability and susceptibility to rot-causing fungi (Lattanzio et al., 1996), especially gray mold caused by *B. cinerea* (Pers.:Fr.) (Wszelaki & Mitcham, 2003). Control of *B. cinerea* is normally carried out by the application of fungicides. There is, however, a growing international concern over the often indiscriminate use of synthetic fungicides on food crops because of the possible harmful effects on human health (Norman, 1988). In addition, public concern and regulatory restrictions about the presence of fungicide residues on crops have emphasized the need to find alternative methods for disease control (Smilanick, 1994).

Microbial biocontrol agents have shown great potential as an alternative to synthetic fungicides for the control of postharvest decay of fruits and vegetables (Wisniewski & Wilson, 1992). Recently, biological control has been developed as an alternative to synthetic fungicide treatment and considerable success has been achieved upon utilizing antagonistic microorganisms to control both preharvest and postharvest diseases (Janisiewicz & Korsten, 2002). Phylloplane yeast *Rhodotorula mucilaginosa* has been reported to control *B. cinerea* on geranium seedlings in combination with fungicides (Buck, 2004). Our research team found that *R. mucilaginosa* showed biocontrol efficacy against blue mold and gray mold of apples caused by *Penicillium expansum* and *B. cinerea* respectively (Li, Zhang, Liu, & Zheng, 2011).

Alternatives to chemical control, particularly biological control, are, however, often less effective than many of the commercial fungicides currently in use. So the efficacy of antagonistic yeasts in controlling of postharvest disease must be enhanced (Janisiewicz & Korsten, 2002). The substances (organic and inorganic additives) have also been applied in combination with biocontrol agents to get beneficial effects (El-Ghaouth, Smilanick, Wisniewski, & Wilson, 2000; Janisiewicz, 1994; Meng, Qin, & Tian, 2010).

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Phytic acid is a natural plant compound that is a simple ringed carbohydrate with six phosphate groups attached to each carbon (Shamsuddin, 2002). Phytic acid originates in most cereal grains, legumes, nuts, zoilseeds, tubers, pollen, spores and organic soils (Febles, Arias, Hardisson, Rodriguez-Alvarez, & Sierra, 2002). Although phytic acid was not given the status of "generally recognized as safe" (GRAS) by the Food and Drug Administration of the United States in their 1995 list, it was used extensively as a food additive outside the U.S. The anti-oxidant or iron chelating properties of phytic acid render this compound a unique and versatile food preservative. Phytic acid is routinely added to meats, fishmeal pastes, canned seafoods, fruits, vegetables, cheese, noodles, soy sauce, juices, bread and alcoholic beverages to prevent product discoloration, increase nutritional quality and prolong shelf-life. By 1997, sodium phytate was listed as a GRAS substance and has been used as a preservative for baked goods in the U.S. (Hix, Klopfenstein, & Walker, 1997). However, to our knowledge, there is no information concerning the effect of a combination of antagonistic yeast and PA on the control of the postharvest diseases of strawberries.

The objectives of this study were to evaluate: (a) the effects of the yeast antagonist *R. mucilaginosa*, used alone or in combination with PA on controlling postharvest gray mold spoilage of strawberries; (b) the effects of PA treatment on the mycelial growth of *B. cinerea*; (c) the effect of PA on the growth of *R. mucilaginosa* in NYDB media and population dynamics on fruit wounds, and (d) the efficacy of *R. mucilaginosa* and PA, used alone or in combination, in controlling of natural spoilage of strawberries.

2. Materials and methods

2.1. Pathogen inoculum

B. cinerea (Pers.:Fr.) was isolated from infected strawberry fruits. The culture was maintained on potato-dextrose agar media (PDA: extract of boiled potatoes, 200 g; glucose, 20 g; agar, 20 g and distilled water, 1000 ml) at 4 °C; fresh cultures were grown on PDA plates at 28 °C before use. Spore suspensions were prepared by removing the spores from the sporulating edges of a 7 days old culture with a bacteriological loop, and suspending them in sterile distilled water. Suspensions were filtrated through four layers of cheesecloth to remove fungal mycelium and spore concentrations were determined with a hemocytometer with the concentration being adjusted as required for different experiments by adding sterile distilled water.

2.2. Antagonist

The yeast antagonist *R. mucilaginosa* was isolated from the surfaces of peach blossom picked in unsprayed orchards. Classical methods based on colony and cell morphologies were used for a preliminary characterization of the yeast. Subsequently, sequence analysis of the 5.8S internal transcribed spacer (ITS) ribosomal DNA (rDNA) region was used to identify the yeast (Li et al., 2010). *R. mucilaginosa* has been shown to be safe in animal testing, including physiology experiments, acute toxicity studies, and the Ames test (our unpublished data). *R. mucilaginosa* isolates were maintained at 4 °C on Nutrient Yeast Dextrose Agar (NYDA) medium containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar (Sangon Co., Shanghai, China), in 1 l of distilled water. Liquid cultures of the yeast were grown in 250 ml Erlenmeyer flasks containing 50 ml of NYD Broth (NYDB) which had been inoculated with a loop of the culture. Flasks were incubated on a rotary shaker at 28 °C for 20 h. Following incubation, cells were centrifuged (TGL-16M Centrifuge, Xiangyi Co., Changsha, China) at 5000 × *g* for 10 min and washed twice with sterile distilled water in order to remove the growth medium. Yeast cell pellets were re-suspended in sterile distilled water and adjusted to an initial concentration of $2–5 \times 10^9$ cells/ml before being adjusted to the concentrations required for the different experiments.

2.3. Fruits

Strawberries (*Fragaria ananassa* Duch.) cultivars 'fengxiang' were harvested early in the morning from the orchard and rapidly transferred to the laboratory. Berries were sorted on the basis of size, color (75% full red color) and absence of physical damage, and were randomly divided into lots of ten fruit. The strawberries were treated immediately, and the temperature at which strawberries were kept before experiment was the room temperature (about 20 °C).

2.4. Effects of R. mucilaginosa in combination with PA on gray mold spoilage of strawberries

The surface of strawberries was wounded with a sterile cork borer (approximately 3-mm-diameter and 3-mm-deep). Each wound was treated with 30 μl of (1) the cell suspensions of *R. mucilaginosa* (1×10^8 cells/ml), (2) *R. mucilaginosa* suspensions (1×10^8 cells/ml) in combination with PA (Sangon Co., Shanghai, China) at the concentration of 2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml and 10 μmol/ml, and (3) sterile distilled water as the control. Two hours later, 30 μl of *B. cinerea* suspensions (1×10^5 cells/ml) was inoculated into each wound respectively. After air drying, the samples were stored in enclosed plastic trays to maintain a high relative humidity (about 95%) and incubated at 20 °C in an incubator (Radford Technology Co., Ltd., Ningbo, China). The percentage of infected fruit was recorded after 3 d inoculation. There were three replicates of 20 fruits for each treatment, and the experiment was conducted twice.

2.5. Effects of R. mucilaginosa in combination with PA on mycelial growth of B. cinerea

The effects of PA on mycelial growth of *B. cinerea* were assayed in PDA. 5-mm-diameter and 5-mm-deep disks were cut from potato-dextrose agar (PDA) plates, then, a 100 μl quantity of 1×10^8 cells/ml washed cell suspension of *R. mucilaginosa* (1×10^8 cells/ml), or *R. mucilaginosa* (1×10^8 cells/ml) in combination with PA at the concentration of 2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml or 10 μmol/ml, or sterile distilled water as the control, respectively, was added into each hole site of PDA plates. After 2 h, 100 μl of 1×10^4 spores/ml suspension of *B. cinerea* was added into each hole. The plates were incubated at 28 °C for 5 d after which the colony diameter of *B. cinerea* was recorded. There were three replicates of 6 plates per treatment, and the experiments were repeated three times.

2.6. Effects of PA on survival of R. mucilaginosa in NYDB

The effects of PA on the growth of *R. mucilaginosa* in NYDB were assayed according to procedures described previously (Zhang et al., 2007). Aliquots of 5 ml NYDB with different concentrations of PA 2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml, 10 μmol/ml and 0 (as a control) in glass tube (180 × 16 mm) were added to 30 μl suspensions of *R. mucilaginosa* (1×10^8 cells/ml), respectively. The number of yeasts was determined by a hemocytometer after 24 h incubation on a rotary shaker at 180 rpm at 28 °C and expressed as Log_{10} cells/ml. Each treatment was replicated three times and the experiment was repeated twice.

2.7. Effects of PA on population growth of R. mucilaginosa in strawberry wounds

Fruit samples were wounded as described above to evaluate the biocontrol to gray mold spoilage of strawberries. The wounds were treated with 30 μl of a suspension of *R. mucilaginosa* at 1×10^8 cells/ml alone or in combination with PA at 4 μmol/ml. The samples were taken at 0, 1, 2 and 3 d at 20 °C or at 0, 3, 6 and 9 d at 4 °C after treatment. The tissue was removed with a sterile cork borer (9-mm-diameter and 10-mm-deep) and ground with a 150-ml Erlenmeyer flasks, by glass rod directly pounding to pieces in 50 ml of sterile 0.85% sodium chloride solution. Serial 10-fold suitable dilutions were made and 0.1 ml of suitable dilution was spread in NYDA. The plates were incubated at 28 °C for 2 d, and the colonies were counted. Population densities of *R. mucilaginosa* were expressed as log10 CFU (colony- forming units) per wound. There were three replicates per treatment and 6 fruits per replicate, and the experiments were repeated twice.

2.8. Effects of R. mucilaginosa and PA on natural spoilage of strawberries

To evaluate the effect of PA enhanced the biocontrol efficacy of *R. mucilaginosa* on development of natural decay of strawberries, intact fruit were inoculated by dipping them into a suspension of (1) *R. mucilaginosa* (1×10^8 cells/ml), (2) a solution of PA (4 μmol/ml), (3) *R. mucilaginosa* (1×10^8 cells/ml) in combination with PA at the concentration of 4 μmol/ml for 60 s, with sterile distilled water as the control, then air dried. The treated fruits were sealed in polyethylene-lined plastic boxes to retain high humidity. Fruits were stored at 4 °C for 20 d followed by 20 °C for 5 d in order to determine disease development under normal shelf-life conditions. Infection rate (the rate of the decay fruits in all treated fruits) was recorded afterward. There were three replicate trials of 10 fruits with a complete randomization in each test and experiments were repeated three times.

2.9. Statistical analyses

The data were analyzed by the analysis of variance (ANOVA) in the statistical program SPSS/PC version II.x. (SPSS Inc. Chicago, Illinois, USA) and the Duncan's multiple range test was used for means separation. The statistical significance was applied at the level $p < 0.05$.

3. Results

3.1. Effects of R. mucilaginosa in combination with PA on gray mold spoilage of strawberries

R. mucilaginosa as stand-alone treatment significantly reduced the disease incidence of gray mold spoilage of strawberries compared with the control after 3 d storage at 20 °C ($p < 0.05$) (Fig. 1). Similarly, *R. mucilaginosa* in combination with PA at all tested concentrations (2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml, 10 μmol/ml) reduced the disease incidence of gray mold spoilage of strawberries. However, the combined treatments of *R. mucilaginosa* and PA at the concentration of 4 μmol/ml and 6 μmol/ml were more effective than *R. mucilaginosa* alone treatment.

3.2. Effects of R. mucilaginosa in combination with PA on mycelial growth of B. cinerea

In the test on PDA plates, *R. mucilaginosa* alone or in combination with PA at all tested concentrations (2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml, 10 μmol/ml) significantly inhibited the growth of *B. cinerea* (Fig. 2). However, the control efficacy of the combined treatments of *R. mucilaginosa* and PA at the concentration of 4 μmol/ml and 6 μmol/ml were better than *R. mucilaginosa* alone treatment.

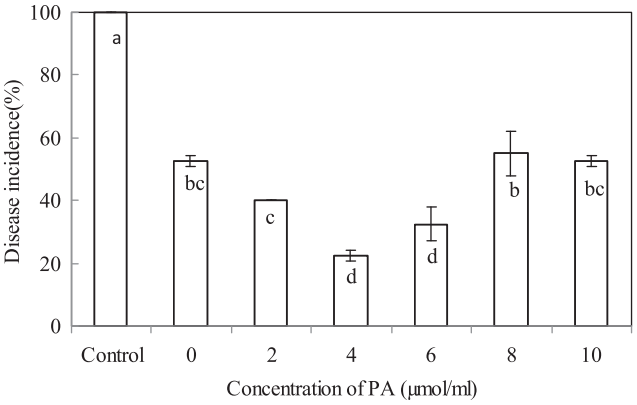

Fig. 1. Effect of *R. mucilaginosa* and PA on gray mold spoilage of strawberries. Fruit treatments are as follow: Control = sterile distilled water, 0 = *R. mucilaginosa* (1×10^8 cells/ml), (2, 4, 6, 8, 10) = *R. mucilaginosa* (1×10^8 cells/ml) + PA at different concentrations (2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml, 10 μmol/ml). Each value is the mean of two experiments. Bars represent standard deviations. Different letter indicates significant differences ($p = 0.05$) according to the Duncan's multiple range test.

3.3. Effects of PA on survival of R. mucilaginosa in NYDB

After 24 h incubation, PA at all tested concentrations (2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml, 10 μmol/ml) significantly increased the growth and survival of *R. mucilaginosa* in NYDB compared with the control (Fig. 3). What's more, PA at the concentrations of 4 μmol/ml, 6 μmol/ml and 8 μmol/ml showed the best enhancement efficacy to the growth and survival of *R. mucilaginosa* in NYDB compared with the other concentrations.

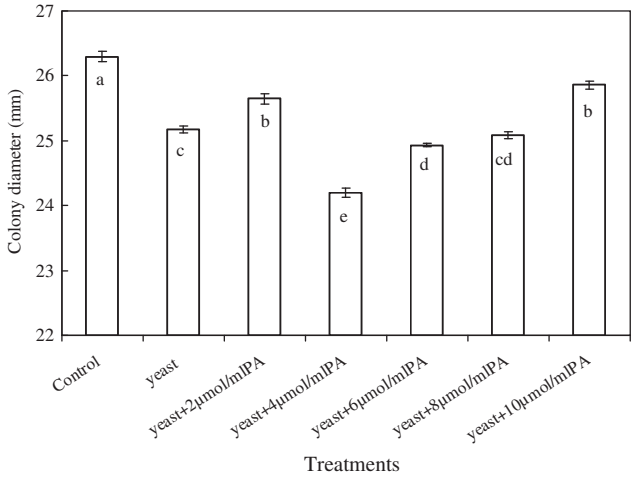

Fig. 2. Effects of *R. mucilaginosa* in combination with PA on mycelial growth of *B. cinerea*. Fruit treatments are as follow: Control = sterile distilled water, yeast = *R. mucilaginosa* (1×10^8 cells/ml), (yeast+2 μmol/ml PA, yeast+ 4 μmol/ml PA, yeast+6 μmol/ml PA, yeast+8 μmol/ml PA, yeast+ 10 μmol/ml PA) = *R. mucilaginosa* (1×10^8 cells/ml)+PA at different concentrations (2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml, 10 μmol/ml). Each value is the mean of two experiments. Bars represent standard deviations. Different letter indicates significant differences ($p = 0.05$) according to the Duncan's multiple range test.

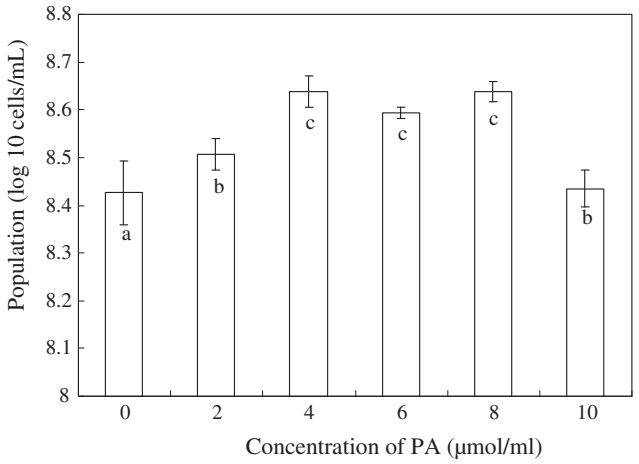

Fig. 3. Effect of PA on the population of *R. mucilaginosa* in Nutrient Yeast Dextrose Broth (NYDB). 0 = NYDB, (2, 4, 6, 8, 10) = NYDB with PA at different concentrations (2 μmol/ml, 4 μmol/ml, 6 μmol/ml, 8 μmol/ml, 10 μmol/ml). The population of *R. mucilaginosa* were measured microscopically after 24 h incubation at 28 °C in NYDB. Bars represent standard errors. Different letter indicates significant differences ($p = 0.05$) according to the Duncan's multiple range test.

3.4. Effects of PA on population growth of R. mucilaginosa in strawberry wounds

The antagonistic yeast *R. mucilaginosa*, no matter it was used only or combined with 4 μmol/ml PA, proliferated rapidly in strawberry wounds, during the first 1 d and stabilized relatively thereafter at 20 °C (Fig. 4(a)). PA slightly increased the population growth of *R. mucilaginosa* in fruit wounds at 20 °C at the first day, but had little influence on the population growth of *R. mucilaginosa* in fruit wounds at 2 d and 3 d. On strawberry wounds kept at 4 °C, the increase in population density of *R. mucilaginosa* was lower than those kept at 20 °C, but continued over 3 d after application of the antagonist until it reached a high level (Fig. 4 (b)). PA slightly increased the population growth of *R. mucilaginosa* in fruit wounds at 4 °C at the whole storage time (3, 6, 9 d after storage).

3.5. Effects of R. mucilaginosa and PA on natural spoilage of strawberries

The results presented in Fig. 5 demonstrate that the application of PA alone did not significantly reduce natural spoilage of strawberries at 4 °C for 20 d followed by 20 °C for 5 d. Whereas, *R. mucilaginosa* as stand-alone treatment significantly reduced natural spoilage of strawberries ($p < 0.05$). The combination of *R. mucilaginosa* and PA was most effective in preventing natural spoilage, decreasing the incidence of fungal spoilage of the fruit to 50% compared to 90% for the untreated controls for fruit stored at 4 °C for 20 d followed by 20 °C for 5 d.

4. Discussion

The use of biocontrol agents to manage postharvest decay of fruit has been explored as an alternative to the use of synthetic fungicides (Wilson & Wisniewski, 1989). Our results showed that *R. mucilaginosa* as stand-alone treatment significantly reduced the disease incidence of gray mold decay of strawberry fruits at 20 °C. The results reported here showed that *R. mucilaginosa* has potential as a biocontrol agent for the control of postharvest gray mold decay of strawberries. However, for general biological control acceptance by growers, efficacy has to be comparable to the level of control provided by conventional fungicides (Obagwu & Korsten, 2003). Achieving such high levels of control is difficult with biological control systems including *R. mucilaginosa*. Therefore, it is important to find effective methods that could increase the control efficacy of antagonists and inhibit pathogenic spoilage.

The induction of mycotoxin production by an oxidative environment has been reported for several post harvest fungi and, furthermore, it has been widely demonstrated that certain oxidants are able to modulate and trigger the biosynthesis of mycotoxins by such fungi (i.e. *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus ochraceus*) (Reverberi et al., 2008). As a consequence, natural anti-oxidants extracted from various plants and fungi have recently been used as novel compounds in the battle against post harvest development of fungi and production of mycotoxins (i.e. aflatoxins, ochratoxin A) (Reverberi et al., 2005; Ricelli et al., 2002; Zjalic et al., 2006). Recently, phytic acid is considered to be an anti-oxidant agent, because it is a potent inhibitor of the iron-catalyzed hydroxy radical formation by chelating the free iron and then blocking it's coordination site (Graf & Eaton, 1990). In our study, the combination treatment of *R. mucilaginosa* with PA at 4 μmol/ml or 6 μmol/ml

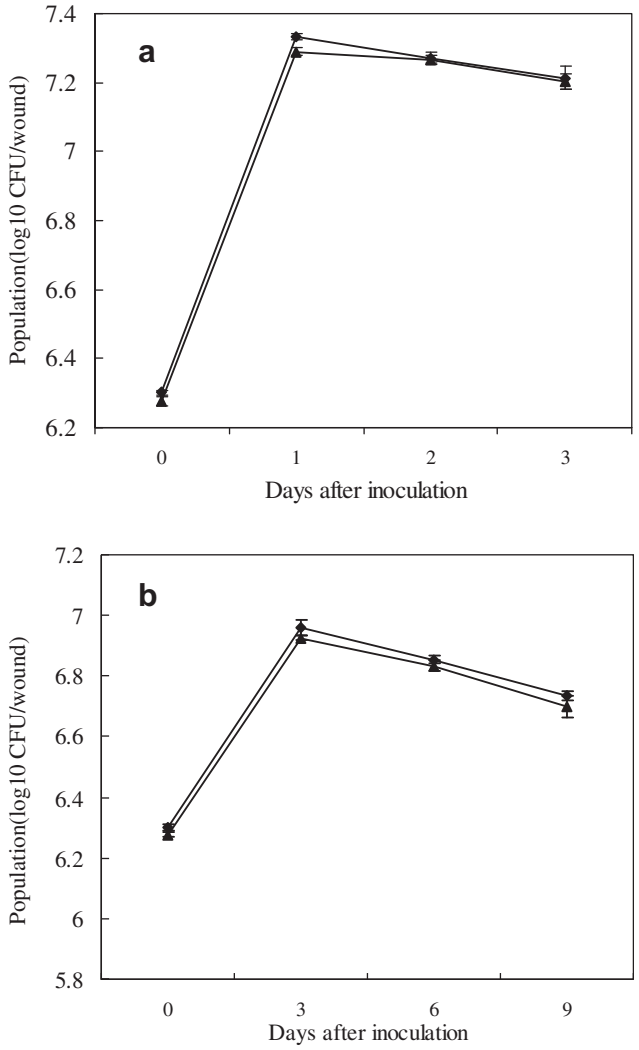

Fig. 4. (a). Effect of PA on population dynamics of *R. mucilaginosa* in wounds of strawberries at 20 °C. Bars represented standard deviations. —▲— *R. mucilaginosa*, —◆— *R. mucilaginosa* + PA at the concentration of 4 μmol/ml. (b). Effect of PA on population dynamics of *R. mucilaginosa* in wounds of strawberries at 4 °C. Other information as in Fig. 4(a).

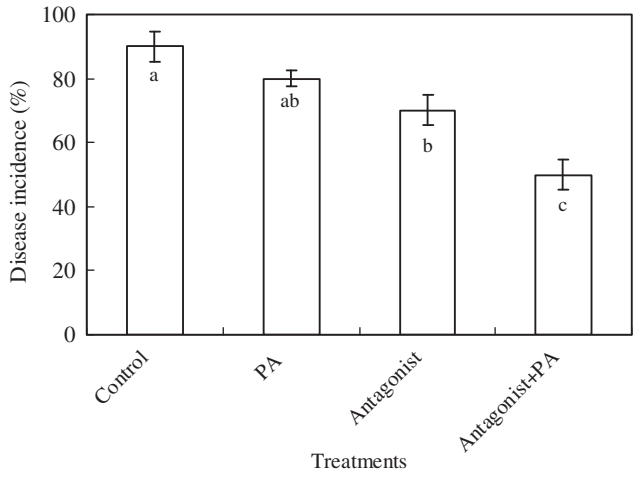

Fig. 5. Effects of PA and *R. mucilaginosa* on natural decay development of strawberries. Control = sterile distilled water, PA = PA at the concentration of 4 μmol/ml, Antagonist = *R. mucilaginosa* (1×10^8 cells/ml), Antagonist + PA = *R. mucilaginosa* (1×10^8 cells/ml) + PA at the concentration of 4 μmol/ml. Each value is the mean of three experiments. Bars represent standard deviations. Different letter indicates significant differences ($p = 0.05$) according to the Duncan's multiple range test.

significantly reduced the disease incidence of gray mold decay of strawberries at 20 °C, and the control efficacy was better than *R. mucilaginosa* as stand-alone. These results suggested that combining PA at some concentrations with *R. mucilaginosa* significantly enhanced the biocontrol activity of *R. mucilaginosa* against gray mold decay of strawberries. The mechanism by which PA enhances the biocontrol efficacy of yeast is complex. This may be attributed to the influence of PA on antagonist, pathogen, and fruit.

Under *in vitro* conditions, *R. mucilaginosa* significantly inhibited the growth of *B. cinerea* on PDA plates, and the control efficacy of the combined treatments of *R. mucilaginosa* and PA at the concentration of 4 μmol/ml and 6 μmol/ml were better than *R. mucilaginosa* alone treatment. In the test of effects of PA on survival of *R. mucilaginosa* in NYDB, PA at all tested concentrations significantly increased the growth and survival of *R. mucilaginosa* in NYDB. It's selectivity in inhibiting the colony diameter of the pathogen but not the growth of the antagonistic yeast may be one of the mechanisms by which PA enhances the biocontrol efficacy of *R. mucilaginosa*.

Our results showed that *R. mucilaginosa* could rapidly colonize and grow in strawberry wounds whether at 20 °C or 4 °C. Such rapid growth in wounds indicated that *R. mucilaginosa* is well adapted to the wound environment in fruit. The biocontrol yeasts have been selected mainly for their capacity to rapidly colonize and multiply in surface wounds, and subsequently to compete the pathogen for nutrients and space (Droby et al., 2002). The nutritional environment at the wound site may be favorable to *R. mucilaginosa*, which rapidly colonizes the fruit tissues and will be competing with the pathogen for nutrients. Our results also showed that PA slightly increased the population growth of *R. mucilaginosa* in fruit wounds at the first day at 20 °C, and slightly increased the population growth of *R. mucilaginosa* in fruit wounds at the whole storage time at 4 °C. When PA was used with *R. mucilaginosa* in fruits, *R. mucilaginosa* multiplied rapidly in wounds at the first stage of storage and, perhaps, consumed available nutrients, which may facilitate biocontrol by nutrient competition. These results suggest that PA enhance the biocontrol efficacy of *R. mucilaginosa* to postharvest diseases of strawberries by facilitating the growth of *R. mucilaginosa* in strawberries and enhancing the activity of nutrient competition. Plant pathogens have evolved a variety of strategies for extracting nutrients from their hosts (Vogel, Raab, Somerville, & Somerville, 2004), and the main mode of action of yeast biocontrol agents has been considered to be competition for space and nutrients (Zhang et al., 2007). When PA was used with *R. mucilaginosa* in fruits, it inhibited the colony growth of the pathogens but not the growth of *R. mucilaginosa*, so, *R. mucilaginosa* multiplied rapidly in wounds and, perhaps, consumed available nutrients, which may facilitate biocontrol by nutrient competition.

In the experiments on efficacy of *R. mucilaginosa* for reducing natural decay development, natural disease incidence of strawberries was significantly reduced by *R. mucilaginosa* following storage at 4 °C for 20 d followed by 20 °C for 5 d. This implies that *R. mucilaginosa* has potential to control a wide range of pathogens under normal shelf-life conditions (including both low temperature and ambient storage). Furthermore, the results from this study showed that the biological control activity of *R. mucilaginosa* against natural decay of strawberries was greatly enhanced by PA compared with *R. mucilaginosa* used alone at 4 °C for 20 d followed by 20 °C for 5 d. In the experiments of effects of *R. mucilaginosa* in combination with PA (4 μmol/ml) on postharvest quality parameters of strawberries, no impaired quality parameters including weight loss, fruit firmness, titratable acidity, browning potential and ascorbic acid were discovered in the fruits under commercial conditions (our unpublished data). *R. mucilaginosa* works as a nutrient competitor, may consume sugar in the face of strawberry fruit, however, no matter it was used only or combined with PA at the concentration of 4 μmol/ml, did not decrease total soluble solids (which composed mainly of soluble sugar) of strawberry fruit (our unpublished data). Since that PA has no risks to humans and to the environment, and that PA is a cheap and widely available natural component, the utilization of PA might be an effective, safe and economic approach to enhance the biocontrol efficacy of *R. mucilaginosa* to postharvest decay of strawberries.

In conclusion, PA at the concentration of 4 μmol/ml could significantly enhance the biocontrol activity of *R. mucilaginosa* against postharvest gray mold decay and natural decay development of strawberries. The mode of action may be involved in its direct inhibition on the colony growth of the pathogens and facilitating the growth of *R. mucilaginosa* in strawberries and enhancing the activity of nutrient competition. Future research will be aimed at developing the technology to be used under large-scale operations and investigating the mode of action of the induction of resistance in strawberries by these treatments.

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